PROPERTIES OF THE BLOOD OF THE SKATE (RAIA OSCILLATA)

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The system developed in the skate for transport of oxygen and carbon dioxide is similar in some respects to that of man. Oxygen combines reversibly with hemoglobin and probably passes in and out of the blood by diffusion. Carbon dioxide, transported as bicarbonate, is prevented from greatly changing the blood reaction by the buffer function of proteins.

If one seeks a more detailed description of the skate's respiratory system, it is found to differ from that of man in many respects. Thus a labile body temperature introduces a degree of freedom in the skate's blood which is absent in the blood of normal man. Other differences depend upon the physical state of the environment. In the one case the blood is separated by a membrane from a moving liquid. From this liquid, oxygen in solution diffuses into the blood stream; into it, carbon dioxide passes directly. In the other case the lung, acting as a buffer between the blood and a variable external environment, maintains air nearly constant in temperature and composition in the ultimate areas where gas exchange takes place. Carbon dioxide passes from the blood, not into a virtual vacuum, as from the gills of the skate, but into a gas phase where the partial pressure of carbon dioxide fluctuates within narrow limits about a mean value of 40 mm. So much can be said by induction; it remains to be seen how well the properties of the skate's blood are adapted to the requirements.

The experimental methods used in this investigation have been described by Dill and Edwards (1931) and need not be discussed here. Two difficulties arose, both related to the character of the erythrocytes. These are very resistant to rupture and in determining oxygen content low values may be obtained because of incomplete hemolysis. When the quantity of saponin in the ferricyanide reagent was tripled, hemolysis was complete and oxygen could be determined satisfactorily. The other difficulty was a consequence of the high metabolic rate of these nucleated cells. After blood is equilibrated and sealed in sampling tubes a rapid decrease in its oxygen content occurs. Usually

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oxygen was determined immediately but occasionally delay was unavoidable. In order to correct for changes in oxygen content, the rate of oxygen consumption was determined at several temperatures.

Since these observations on metabolic rate of the blood may be of some interest in themselves they have been tabulated in Table I. In this experiment the blood had been saturated at a temperature of 10.5° with an oxygen partial pressure of 200 millimeters. Assuming the same solubility as in human blood of the same water content, dissolved oxygen amounted to 0.82 volume per cent and combined oxygen, 4.74 volumes per cent. With this information and the data of Table I and of Fig. 1 on the metabolic rates at 0, 20 and 40°, one can calculate the oxygen consumption of any specimen of blood

TABLE I

Oxygen Consumption by Skate's Blood

Initial oxygen content = 5.56 volumes per cent

Time *min.*	Oxygen Content *vol. per cent*		
	0° C.	20° C.	40° C.
21			4.70
96			1.88
115			1.64
140		3.70	
375		1.37	
1000	4.29		

within a wide range of conditions. It appears from Fig. 2 that the metabolic rate is not a linear function of the reciprocal of the absolute temperature. The observations are too few in number, however, to define this curve precisely.

Observations have been made on the oxygen dissociation curves of three specimens of blood (two of which were composited from several skates) at four temperatures. A preliminary experiment was carried out in the usual way with variable oxygen pressures and with carbon dioxide partial pressures ranging from 10 to 100 millimeters. Since the blood has about one-half the buffer value of human blood it was supposed that this range of carbon dioxide pressures would have a greater effect on the position of the oxygen curves than in human blood. On the contrary, the effect was too small to be evaluated. A second experiment (Blood B; temperature 10.4°; Table II) was carried out with a greater range in carbon dioxide pressures,—from 0.5 to 140 millimeters. With this extreme range there appeared to be a small decrease in affinity for oxygen with increasing acidity.

TABLE II

Equilibrium Values for Oxygen Absorption

	pCO_2	pO_2	Total O_2	HbO_2
	mm. Hg	*mm. Hg*	*vol. per cent*	*per cent of capacity*
Blood A. HbO_2 capacity 5.68 vol. per cent	0.8	12.5	0.22	4
Temperature 37.5°	1.0	48.2	1.54	25
	0.8	128	3.97	64
	1.3	248	5.29	82
Temperature 25°	0.8	14.6	0.41	6
	0.7	31.3	1.79	30
	0.7	84.6	4.53	75
	1.0	100	4.55	75
Temperature 0.2°	0.5	2.4	0.60	10
	0.6	10.8	2.80	48
	0.5	13.8	3.65	62
	0.7	24.3	4.94	85
	0.7	48.6	5.68	96
	1.5	150	6.43	100
Blood B. HbO_2 capacity 5.54 vol. per cent	0.7	3.6	0.34	6
	0.7	5.3	0.32	6
Temperature 10.4°	0.5	8.1	1.00	18
	1.5	16	2.20	39
	0.5	26.5	3.62	64
	0.6	40.1	4.56	79
	0.8	58.8	5.15	89
	1.1	76.8	5.54	95
	2.3	190	6.30	100
	10.7	25.8	2.68	47
	140	23.2	2.29	40
	141	33.0	3.07	53
	137	40.7	3.41	59
Blood C. HbO_2 capacity 4.18 vol. per cent	2.2	108	2.60	56
Temperature 37.5°	2.7	254	4.13	84
Temperature 25°	0.6	23.2	1.24	28
	0.5	48.9	2.50	56
	0.5	83.1	3.19	70
	0.6	108	4.04	89
	1.0	165	4.49	95
	1.6	212	4.75	98
Temperature 10.4°	0.9	18.8	2.49	58
	0.5	22.3	2.42	56
	0.6	38.9	3.14	71
	2.2	167	4.95	100

This effect is represented quantitatively in Fig. 3. Ordinarily it is convenient to express the relation between position of the oxygen dissociation curve and pH by such an equation as

$$\log (X_{50}) = a(\mathrm{pH}) + b,$$

where X_{50} represents the partial pressure of oxygen when the blood is 50 per cent saturated with oxygen, and the term a, the slope, is a measure of the rate of change of affinity of the blood for oxygen with rate of change in pH. This equation cannot be applied to

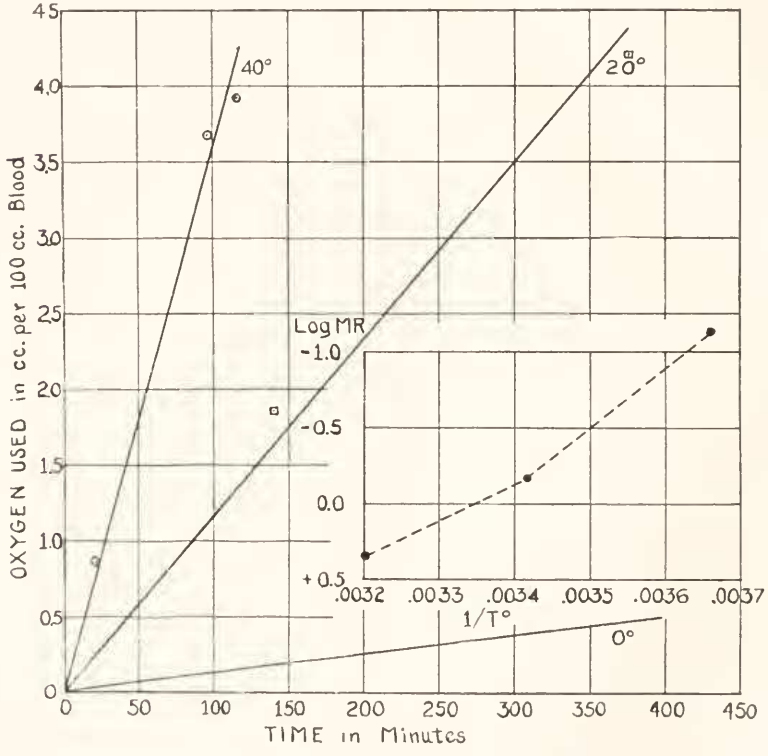

FIG. 1. Rate of oxygen consumption of normal skate's blood at temperatures of 0, 20, and 40°.

FIG. 2. (Inset.) Logarithm of the metabolic rate of skate's blood (oxygen used in cc. per hr. per 100 cc. blood) as a function of the reciprocal of the absolute temperature.

our data on skate's blood since we do not have direct determinations of pH nor knowledge of the value of pK′ for serum or for cells. For our purposes, however, it is enough to use calculated values for log $[(BHCO_3)_b/(H_2CO_3)_b]$. This quantity differs from pH by a

constant (or nearly constant) amount and hence the slope a will be essentially the same as though pH values were used. The curves for blood of the crocodile and of man shown in this figure are from Dill and Edwards (1931).

The curves for blood of the crocodile and for man are much steeper than for that of skate's blood. The contrast is greatest in the acid range; in fact within the range which corresponds to pCO_2 values from 10 to 140 mm. there is almost no change in position with change in reaction. However, the skate does not normally function within these limits but, as will be shown below, at a carbon dioxide pressure

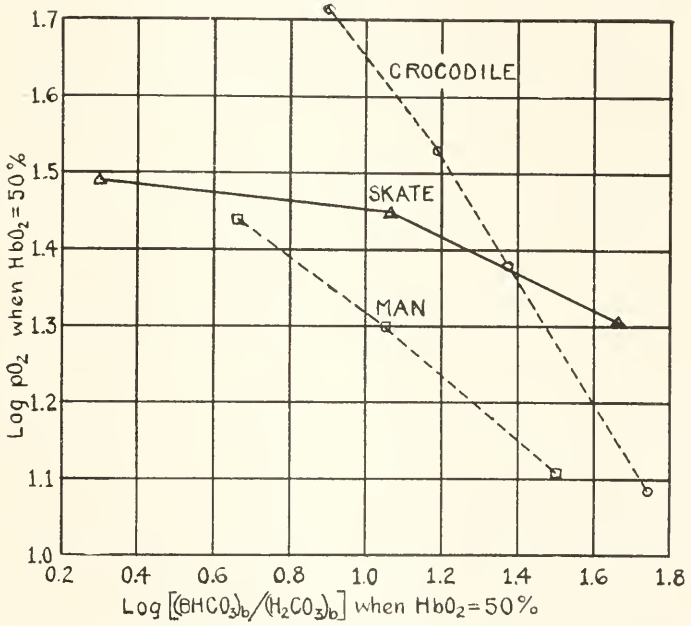

FIG. 3. Position of the oxygen dissociation curves (log pO_2 when $HbO_2 = 50$ per cent) in relation to log $(BHCO_3)/(H_2CO_3)$.

of one or two millimeters. In this range there is a distinct acid effect on oxygen affinity; here the slope a is roughly one-half as great as in man and one-fourth as great as in the crocodile. The blood of the skate is like that of *Urechis*, studied by Redfield and Florkin (1931), in the acid range but quite different when the reaction is more alkaline. They have found that the affinity of *Urechis* blood for oxygen is unaffected by change in hydrogen ion concentration when pCO_2 is varied from 0.5 to 92 millimeters. It would be interesting to speculate on the possible significance of these relationships in connection with structure of the hemoglobin molecule. However, we must remember

that the environment of hemoglobin is very different in these cases. It will be recalled that an abnormal value for a similar relationship was found in man in diabetic coma (Dill and others, 1929). Further discussion of this question had better wait, therefore, until it is possible to prepare these hemoglobins in the pure state and study them under strictly comparable conditions.

The data given in Table II have been used to construct the oxygen dissociation curves of Fig. 4. These have been drawn as members of the same family of curves, and aside from a few bad results the fit accords with this assumption. The effect of temperature on the affinity of blood for oxygen has been shown graphically for human blood by Brown and Hill (1923). Use has been made of their data and that of Fig. 4 in Fig. 5. This comparison indicates that q, the heat of reaction of 1 gram molecule of hemoglobin with n gram mole-

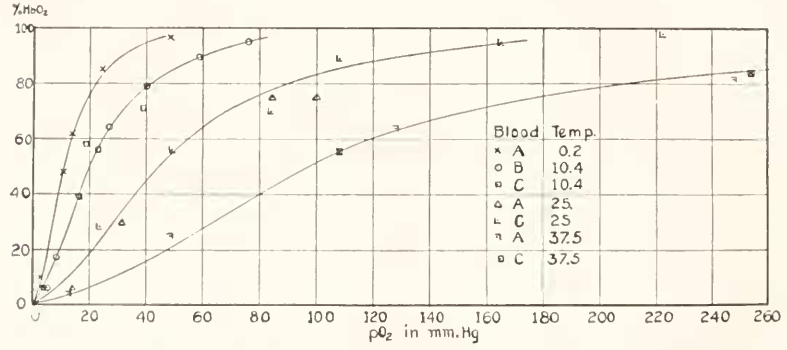

FIG. 4. Oxygen dissociation curves of skate's blood at temperatures of 0.2 10.4, 25 and 37.5° C. when $pCO_2 = 1 \pm 0.5$ mm.

cules of oxygen, is the same for the blood of the skate and of man. The significance of n remains somewhat obscure, but it is useful in characterizing the slope of the oxygen dissociation curve when $\log pO_2 = (f) \log Hb/HbO_2$. It has the value of 2.2 in man and in skate's blood it is slightly smaller, *viz.*, 2.0. If we accept the definition of n given by Brown and Hill, it follows that the value for Q, the heat evolved when 1 gram molecule of oxygen combines at constant volume with hemoglobin, is about the same as in human blood.

One must have a description of the carbonic acid dissociation curve of blood in order to understand the conditions under which carbon dioxide is excreted through the gills. It is known from the work of Collip (1920), confirmed by others (Kokubo, 1927 and Smith, 1929), that the carbonic acid content of selachian blood is no more than 10 to 12 volumes per cent. Accordingly the carbonic acid

dissociation curves have been studied over a low range of partial pressures. The curves for oxygenated blood are shown for temperatures of 10.4° (body temperature [2]) 25° and 37.5° in Fig. 6. The experimental procedure was simplified by the fact that, as in human blood, the relation of log (pCO_2) to log (Total CO_2) is linear or nearly so. Hence it was only necessary to determine a few points and fit the best straight line. The smoothed values were then transformed to the more familiar system of coördinates used in Fig. 6.

The alkaline reserve of blood, as suggested by Van Slyke and Cullen (1917), is most accurately defined by the bicarbonate content of arterial blood. When this is impractical, their method of equilibrating plasma of venous blood with alveolar air gives approximately correct values for human blood. The application of this method to fish blood, as by Collip (1920) and Kokubo (1927) does not define the alkaline reserve of fish blood but merely the carbon dioxide

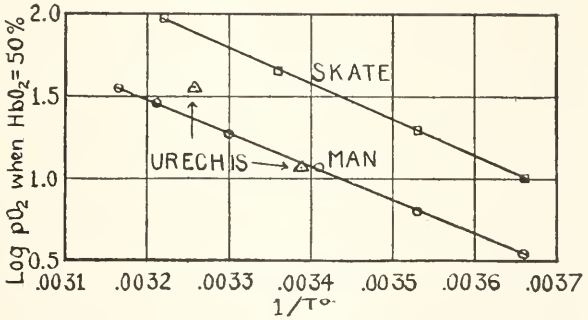

FIG. 5. Position of oxygen dissociation curves (log pO_2 when HbO_2 = 50 per cent) as a function of the reciprocal of the absolute temperature. The data for man are from Brown and Hill (1922–3) and for *Urechis*, from Redfield and Florkin (1931). In each case the oxygen is expressed in terms of partial pressures at the actual temperatures involved. The partial pressures of carbon dioxide were approximately 40 mm. for man, 1 for the skate and 12 for *Urechis*.

content at a partial pressure of carbon dioxide which is possibly twenty times greater than that of blood *in vivo*. It is probable that the values given by Collip for carbon dioxide content of blood equilibrated with atmospheric air measure the alkaline reserve more accurately than when alveolar air was used.

Having attained a description of oxygenated blood there remained to determine the effect of oxygenation on the position of these curves. There are several *a priori* reasons for supposing that this effect is too

[2] Body temperature was observed by rectum. It was maintained approximately constant by circulating sea water. Blood drawn at 10.4° and equilibrated at 25° and at 37.5° does not necessarily reflect the properties of blood drawn from animals acclimated to these higher temperatures. Possibly the blood would be altered in respect to available base and in other respects by change in body temperature.

small to be measurable in skate's blood. These are: (*a*) the hemoglobin is one-fourth its concentration in normal human blood and the effect of oxygenation on the carbonic acid dissociation curves of blood will be reduced accordingly; (*b*) the alkaline reserve is lower and the distance between the curves will on this account be low, as may be

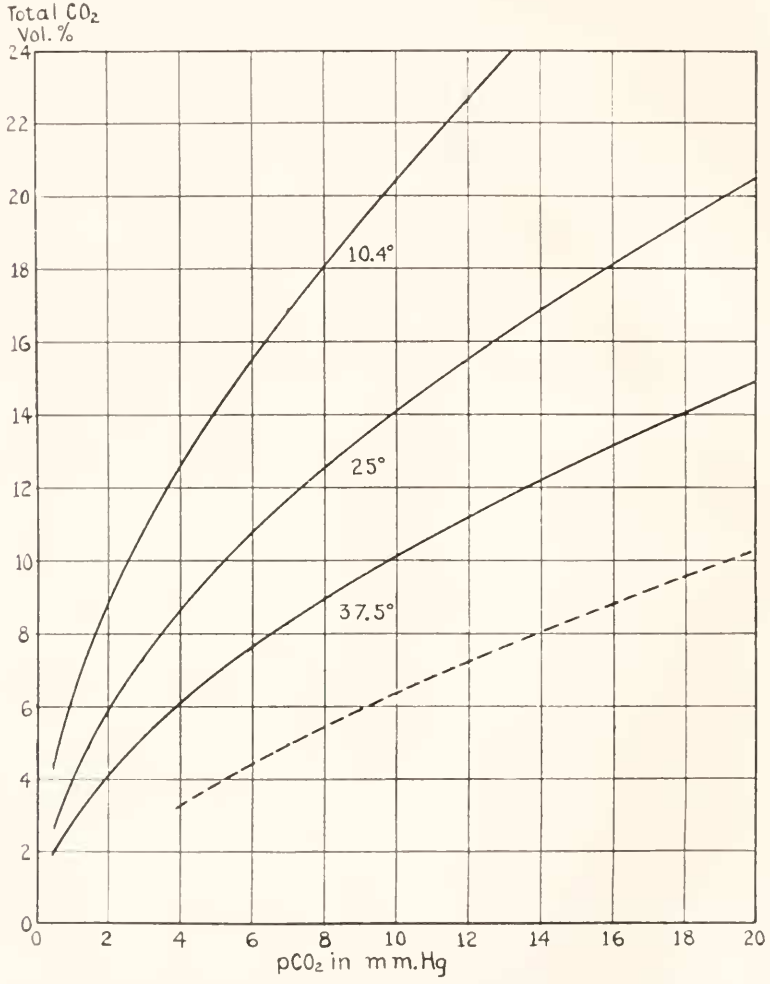

FIG. 6. Carbonic acid dissociation curves of skate's blood, body temperature 10.4°; equilibration temperatures, 10.4, 25 and 37.5°. The broken line corresponds to human blood at 37.5° in terminal chronic nephritis.

seen from the curves for human blood in diabetic coma (Dill and others, 1929); (*c*) the effect of acid on the oxygen dissociation curves is much less in skate's blood than in human blood (see Fig. 3); accordingly

the effect of oxygen on the base bound by hemoglobin should be correspondingly less. For all these reasons taken together it would appear that oxygenation should have little effect on the carbonic acid dissociation curves and in fact several experiments, including one on concentrated blood, revealed no significant difference between the curves of oxygenated and of reduced blood.

It appears, then, that in the skate carbon dioxide is transported principally by virtue of buffering properties of blood proteins and we shall now direct our attention to that subject. It will be convenient to consider first the buffer value of separated plasma. The results of experiments in which the carbonic acid dissociation curves were used to calculate buffer value of plasma specimens are shown graphically

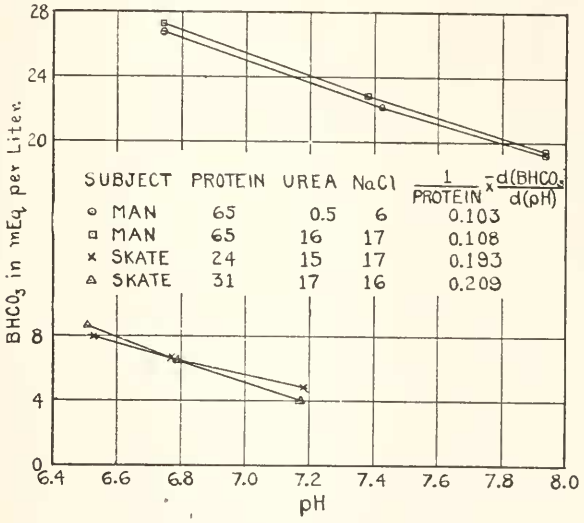

FIG. 7. Buffer value of skate's plasma and human plasma compared. Enough salt and urea was added to one specimen of human plasma to simulate skate's plasma in respect to those constituents. Concentrations of protein, urea and salt are in grams per liter.

in Fig. 7. Van Slyke's measure of buffer value is the rate of change of base bound with rate of change in pH:

$$\beta = \frac{dB}{d(\text{pH})}.$$

These curves define the buffering capacity of plasma, but since this is due principally to protein, it has been divided by the protein concentration in each case. The calculations inserted in Fig. 7 indicate an unusually high value for the buffer value of plasma protein of the skate. To determine the extent to which this is due to the high

concentrations of urea and of sodium chloride in skate's plasma, a human plasma was prepared with concentrations of these substances typical of the skate. The effect of this modification proved to be very small and it follows that the proteins of the skate's plasma have, per gram, about twice the intrinsic buffering value of human plasma proteins. It will be noted that the protein concentration in the skate's plasma is from one-third to one-half as great as in human plasma. In effect, then, the buffer value of plasma per unit volume is almost equal in the two species.

It is possible to compare the buffer value of skate's whole blood with that of human blood by reference to the chart of Henderson and associates (1930, Fig. 3). The observations given in Table III are

TABLE III

Buffer Value of Oxygenated Skate's Blood at 37.5°

Blood	HbO_2 Capacity	CO_2 Capacity at $pCO_2 = 40$ mm.	$\triangle CO_{2_{60-30}}$		
			Observed	Calculated from human blood *	Ratio
	mEq. per l.	*mEq. per l.*	*mEq. per l.*	*mEq. per l.*	
Normal	2.0	8.4	3.3	3.1	1.1
Concentrated	4.7	7.0	3.15	3.2	1.0

* These values are calculated from the empirical chart of Henderson and associates (1930, Fig. 3).

based on one specimen of normal blood from the skate and one concentrated specimen obtained by centrifuging normal blood and removing about one-half the plasma. The comparison indicates that skate's blood has about the same buffer value as human blood of the same oxygen-combining capacity. Since the ratio of cell proteins to oxygen-combining capacity is about one-half greater in the skate than in man, it appears that the buffer value of cell proteins per unit weight is much less in the skate than in man.

This information regarding the physicochemical properties of skate's blood constitutes a suitable basis for study of changes in the respiratory cycle. The additional observations required are the oxygen and carbon dioxide contents of arterial and venous blood. We succeeded in obtaining for this purpose blood from the dorsal aorta and from the conus arteriosus while sea water at body temperature (9 to 10° C.) was being circulated over the gills. By application of the data thus obtained to the carbonic acid and oxygen dissociation

curves it is possible to calculate the partial pressure of carbon dioxide and of oxygen in the blood *in vivo*. This and other calculations are shown in Table IV where a comparison also is made between respiratory changes in the skate and in man in terminal chronic nephritis (Henderson and others, 1927), a state which approximates in many respects to that of the normal skate.

The data given in Table IV have several points of interest. The

TABLE IV

Comparison of the Skate with Man in Terminal Nephritis

	Skate *	Man †
Body temperature, ° *C.*	10.4	37.5
Cell count, *million per mm.*^{3}	0.2	1.0
Red cell volume, *cc. per 100 cc.*	20.0	14.7
Oxygen capacity, *vol. per cent*	6.00	5.60
Free oxygen, arterial blood, *vol. per cent*	0.32	0.28
Combined oxygen, arterial blood, *vol. per cent*	5.58	5.32
Combined oxygen, arterial blood, *per cent of capacity*	93	95
Free oxygen, venous blood, *vol. per cent*	0.07	0.06
Combined oxygen, venous blood, *vol. per cent*	1.91	1.62
Combined oxygen, venous blood, *per cent of capacity*	32	29
CO_2 content, arterial blood, *vol. per cent*	7.70	8.61
CO_2 content, venous blood, *vol. per cent*	10.84	11.75
CO_2 transport, *vol. per cent*	3.14	3.14
O_2 transport, *vol. per cent*	3.92	3.92
pCO_2, arterial blood, *mm. Hg*	1.3	15
pCO_2, venous blood, *mm. Hg*	2.6	23
pO_2, arterial blood, *mm. Hg*	70	110
pO_2, venous blood, *mm. Hg*	14	27
pH_s, arterial	7.82 ‡	7.00
pH_s, venous	7.67 ‡	6.95
ΔpH_s	0.15	0.05

* While these data given for the skate are based on a specimen of arterial blood from one skate and of venous blood from another, observations on other individuals have verified the approximate accuracy of the figures given in the table.

† These values have been obtained directly or by calculation from the study of terminal nephritis by Henderson and associates (1928). The values for carbon dioxide and oxygen transport have been arbitrarily made equal to the observed values in the skate. Associated changes in dependent variables have been read from the alignment chart for blood in nephritis.

‡ These values for pH_s are calculated on the assumption that $pK'_s = 6.24$ under the experimental conditions. The absolute values for pH_s may be incorrect, but the value for ΔpH_s will not be affected.

striking difference as compared with man is in the carbon dioxide partial pressure. Man in terminal nephritis has a greatly increased rate of pulmonary ventilation but the partial pressure of carbon dioxide cannot be kept below 15 mm.; the skate, on the contrary, keeps the carbon dioxide pressure below 2 mm. in arterial blood. The steep character of the carbonic acid dissociation curve in this

range makes possible the transport of 3 volumes per cent of carbon dioxide with a change in its partial pressure of only 1.3 millimeters. Another point of interest is the large change in pH of skate's blood. This is no doubt related to the greater effect in human blood of oxygenation on base bound by hemoglobin.

These facts are of particular interest in connection with the equilibrium between blood and air in respect to oxygen and carbon dioxide. The oxygen dissociation curve of skate's blood at 10.4° is approximately the same as man's at 37.5° and the oxygen tension in sea water is approximately the same as in air. The fact that arterial blood of the skate has about the same percentage saturation with oxygen as that of man indicates that the adequacy of oxygen transfer is approximately the same in the two species.

It has been shown by Bock and Field (1924) that in man the carbonic acid pressure is about the same in alveolar air as in arterial blood, most of the differences in partial pressure being within $\pm$ 1 millimeter. It is now possible to say that the partial pressure of carbon dioxide in the arterial blood of the skate exceeds that in water passing over the gills by no more than 1 or 2 millimeters. Collip has suggested that it is possible that a steep pressure gradient exists "*between the dissolved carbon dioxide in the blood on the one side, and in the sea water on the other.*" Such may be true of some bony fishes but it is not true of the skate.[3] His argument is as follows:

"As the hydrogen ion concentration of sea water is in most instances lower than that obtaining in the blood of marine forms and as the bicarbonate content of the latter is much higher than that of the former it is evident that the amount of the dissolved carbon dioxide in the blood or body fluids of marine forms must be considerably greater than that occurring in sea water. The tension of carbon dioxide in the blood of marine forms must also be proportionately higher than that in sea water."

This argument is sound provided one assumes that sea water is in equilibrium with atmospheric air in respect to free carbon dioxide. It may be misleading, however, because of the use of the word "considerably." Let us make a specific comparison of skate's blood and sea water:

	$BHCO_3$, *vol. per cent*	pCO_2, *mm. Hg*
Sea water	5	0.2
Arterial blood of the skate	8	1.4

[3] In advance of its publication, we have had the privilege of reading the paper by Root (*Biol. Bull.*, 61: p. 427), on the respiratory function of the blood of marine fishes. His single observation on arterial blood of *Prionotus carolinus* shows substantially the same pressure gradient of carbon dioxide from blood in the gills to sea water as we have found in the skate.

Assuming the same pK′, the blood of the skate will be more acid by 0.64 pH units. It is true that the ratio of carbon dioxide pressures is 7 to 1 but pressure gradient depends not on the ratio but on the difference in pressure. This difference, 1.2 mm., is small,—of the same order as in man.

Summary

In the acid range, carbon dioxide pressure has almost no effect on affinity of skate's whole blood for oxygen. In the physiological range the effect is appreciable but still only one-half as great as in man. No difference was discerned between the carbon dioxide dissociation curves of oxygenated and of reduced blood. This was partly due to the facts that the hemoglobin concentration is one-fourth as great as in man and that the carbonic acid-combining capacity (when $pCO_2 = 40$ mm.) is less than in man.

The effect of temperature on the oxygen dissociation curves is identical with that found by Brown and Hill (1923) for human blood but somewhat different from that found by Redfield and Florkin (1931) for *Urechis* blood.

The buffer value of plasma proteins is about twice as great, per unit weight, as that of human plasma proteins. Since the concentration of protein in skate's plasma is one-third to one-half as great as in human plasma, it follows that the buffer value of plasma of the two species is about the same. Buffer value of whole blood is nearly equal to that of human blood of the same oxygen-combining capacity.

Transfer of gases between the blood and the external medium takes place under conditions which are quite different from those in the lungs of man. Nevertheless arterial blood is about equally saturated with oxygen in the two species. The absolute values for carbon dioxide pressure in man and the skate are very different because the blood of the skate is exposed to a virtual vacuum in respect to carbon dioxide. The pressure head of carbon dioxide from blood to the external medium, however, is of the same order of magnitude, about 1 mm. in each species. The supposition that there is a steep pressure gradient in respect to carbon dioxide in such a marine species as the skate is incorrect.

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